Environmental Testing Solutions, Inc.

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March 31, 2010

Dr. Rick Sherrard
Tennessee Valley Authority
1101 Market Street, PSC 1X-C
Chattanooga, TN 37402

RE: ETS PROJECT NUMBER: 6072

Dear Dr. Sherrard:

Enclosed are toxicity test results for samples in support of the **Kingston Fly Ash Recovery Project** received by Environmental Testing Solutions, Inc. on March 24, 2010.

All unusual observations or deviations from standard test protocols are documented on the laboratory bench sheets. If you have any questions concerning these results, please feel free to contact me.

Sincerely,

Jim Sumner
Laboratory Director

CC: Dr. William Rogers, Tennessee Valley Authority

ENVIRONMENTAL TESTING SOLUTIONS, INC.
TOXICITY TEST REPORT

INTRODUCTION / EXECUTIVE SUMMARY

Report Date: March 31, 2010
ETS Project #: 6072

1. Client: Tennessee Valley Authority
2. Study: Kingston Fossil Plant, Fly Ash Recovery Project
3. Samples Tested: Stilling Pond Outfall 001, Emory River Dredge Plume, Unaffected Emory River Water (ERM 12.2)
4. Date Samples Received: March 24, 2010

METHODS SUMMARY (see Appendix A for Additional Test Information)

Samples:

1. Sample Type:

   Grab samples of Emory River Dredge Plume and ERM12.2 and 24-hour composite samples of Stilling Pond Outfall 001 were collected.

2. Sample Transportation, Storage, and Manipulation:

   Samples were placed in ice chests on ice immediately after collection, where they remained during transport to Environmental Testing Solutions, Inc. by private courier. All samples were received at < 6.0°C and were refrigerated at < 6.0°C when not in use.

   Aliquots of these samples, needed to prepare all test dilutions, were warmed to test temperature (25.0 ± 1.0°C) in a warm water bath immediately prior to use.

   Sufficient volumes of each dilution were prepared to split the test concentrations between the Daphnid and fathead minnow tests.

   Aliquots of each dilution were also UV treated. Fish pathogens present in upstream river water have been the suspected cause of test interferences (anomalous dose response and high variability among replicates) in previous toxicity testing at the Kingston Fossil Plant. These aliquots were UV-treated through a 40-watt Smart® UV Sterilizer (manufactured by Emperor Aquatics, Inc.) for 2 to 5 minutes (dependent on sample turbidity).

Test Organisms:

		Pimephales promelas	*Ceriodaphnia dubia*
1.	Source:	Aquatox, Inc.	In-house Cultures
2.	Age:	< 24-hours old	< 24-hours old

Test Conditions Summary:

1.	Test Type/Conditions:	Static Acute, Renewal at 48-hours	Static Acute, Renewal at 48-hours
2.	Test Duration:	96-hours	96-hours
3.	Test Temperature:	25.0 ± 1.0°C	25.0 ± 1.0°C
4.	Light Quality:	Wide-spectrum fluorescent lighting	Wide-spectrum fluorescent lighting
5.	Light Intensity:	50 – 100 ft-c	50 – 100 ft-c
6.	Photoperiod:	16-hours light, 8-hours dark	16-hours light, 8-hours dark
7.	Test Chamber Size/Type:	500 mL plastic disposable cup	40 mL polypropylene cups
8.	Test Solution Volume:	200 mL	35 mL
9.	Number of Replicates:	5	5
10.	Number of Organisms per Replicate:	10	5
11.	Number of Organisms per Test Concentration:	50	25
12.	Feeding regime:	Fed newly hatched *Artemia* in holding prior to test initiation and 2-hours prior to test solution renewal at 48-hours.	Fed YWT and *Selenastrum* in holding prior to test initiation and 2-hours prior to test solution renewal at 48-hours.
13.	Aeration:	None	None
14.	Control / Dilution Water:	Unaffected Emory River Water (ERM 12.2)	Unaffected Emory River Water (ERM 12.2)
15.	Laboratory QC:	Moderately Hard Synthetic Water	Moderately Hard Synthetic Water
16.	Test Chamber Cleaning:	None	None
17.	Test Concentrations (%):	100, 50, 25, 12.5, 6.25, 0 (river control), MHSW	100, 50, 25, 12.5, 6.25, 0 (river control), MHSW
18.	Sample Holding Time:	First use ≤ 36-hours	First use ≤ 36-hours
19.	Endpoints:	Survival	Survival
20.	Test Acceptability Criteria:	≥ 90% survival in river control and negative control	≥ 90% survival in river control and negative control
21.	Physical / Chemical Measurements:	Alkalinity, hardness, and total residual chlorine were measured in each full-strength sample tested. Daily temperatures were measured in one replicate for each test concentration. Pre-exposure test solutions were analyzed at test initiation and at the 48-hour renewal for pH, conductivity, and dissolved oxygen. Post-exposure test solutions were analyzed daily for pH and dissolved oxygen.	
22.	Statistics:	Statistics were performed according to methods prescribed by EPA using ToxCalc version 5.0.23F statistical software (Tidepool Scientific Software, McKinneyville, CA).	

TOXICITY TEST RESULTS

1. Sample: Emory River Dredge Plume (see Appendix B for ToxCalc™ Statistics Reports)

   Collection Date: March 23, 2010

   Test Dates: March 24 – 28, 2010

   96-hour Survival:

   Non-treated Sample:

   Ceriodaphnia dubia: NOEC = 100%

   Pimephales promelas: Invalid test due to mortality in the Unaffected Emory River Water Control. Mortality was attributed to pathogenic bacteria and fungus.

   UV-treated Sample:

   Pimephales promelas: NOEC = 100%

2. Sample: Stilling Pond Outfall 001 (see Appendix C for ToxCalc™ Statistics Reports)

   Collection Date: March 23, 2010

   Test Dates: March 24 – 28, 2010

   96-hour Survival:

   Non-treated Sample:

   Ceriodaphnia dubia: NOEC = 100%

   Pimephales promelas: NOEC = 100%

   UV-treated Sample:

   Pimephales promelas: NOEC = 100%

APPENDIX A
ADDITIONAL TOXICITY TEST INFORMATION

DEVIATIONS / MODIFICATIONS TO TEST PROTOCOL

1. *Pimephales promelas*
   Minnow were < 24-hours old at test initiation.
2. *Ceriodaphnia dubia*
   None

DEVIATIONS / MODIFICATIONS TO PRETEST CULTURE OR HOLDING OF TEST ORGANISMS

1. *Pimephales promelas*
   None
2. *Ceriodaphnia dubia*
   None

PHYSICAL AND CHEMICAL METHODS

1. Reagents, Titrants, Buffers, etc.: All chemicals were certified products used before expiration dates.
2. Instruments: All identification, service, and calibration information pertaining to laboratory instruments is recorded in calibration and maintenance logbooks.
3. Temperature was measured by SM 2550 B.
4. Dissolved oxygen was measured by SM 4500 O G.
5. The pH was measured by SM 4500 H+ B.
6. Conductance was measured by SM 2510 B.
7. Alkalinity was measured by SM 2320 B.
8. Total hardness was measured by SM 2340 C.
9. Total residual chlorine was measured by ORION Electrode Method 97-70.

QUALITY ASSURANCE

Toxicity Test Methods: All phases of the study including, but not limited to, sample collection, handling and storage, glassware preparation, test organism culturing/acquisition and acclimation, test organism handling during test, and maintaining appropriate test conditions were conducted according to the protocol as described in this report and EPA-821-R-02-012. Any known deviations were noted during the study and are reported herein.

REFERENCE TOXICANT TESTS (reference toxicant data is available upon request)

1.	Test Type:	96-hour acute tests with results expressed as LC_{50} values in g/L KCl or NaCl.
2.	Standard Toxicant:	Potassium Chloride (KCl crystalline) for *Pimephales promelas*. Sodium Chloride (NaCl crystalline) for *Ceriodaphnia dubia.*
3.	Dilution Water Used:	Moderately hard synthetic water.
4.	Statistics:	ToxCalc software Version 5.0 was used for statistical analyses.

REFERENCES

1. USEPA. Short-Term Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, EPA-821-R-02-012 (October 2002).
2. Standard Methods for the Examination of Water and Wastewater, 21st Edition, 2005.
3. Quality Assurance Program: Standard Operating Procedures, Environmental Testing Solutions, Inc.

APPENDIX B

ToxCalc™ v5.0.23F Statistics Report
for *Ceriodaphnia dubia* and *Pimephales promelas*
96-hour Acute Toxicity Tests of TVA Kingston Fossil Plant, Emory River Dredge Plume
March 24 – 28, 2010

Environmental Testing Solutions, Inc. Project # 6072

Ceriodaphnia dubia 96-Hour Acute Toxicity Test for Non-treated Emory River Dredge Plume March 24 – 28, 2010

Acute Daphnid Test-96 Hr Survival

Start Date:	3/24/2010	Test ID:	6072	Sample ID:	KIF, Emory River Dredge Plume
End Date:	3/28/2010	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	Non-treated Grab
Sample Date:	3/23/2010	Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	CD-Ceriodaphnia dubia
Comments:	**1 Dredge Plume grab sample for day 0 (initiation) and day 2 (renewal)**				

Conc-%	1	2	3	4	5
MHSW-Control	1.0000	1.0000	1.0000	1.0000	1.0000
ERM-Control	1.0000	1.0000	1.0000	1.0000	1.0000
6.25	1.0000	1.0000	1.0000	1.0000	1.0000
12.5	1.0000	1.0000	1.0000	1.0000	1.0000
25	1.0000	1.0000	1.0000	1.0000	1.0000
50	1.0000	1.0000	1.0000	1.0000	1.0000
100	1.0000	1.0000	1.0000	1.0000	1.0000

			Transform: Arcsin Square Root					Rank	1-Tailed
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical
MHSW-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	5		
ERM-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	5		
6.25	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	5	27.50	16.00
12.5	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	5	27.50	16.00
25	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	5	27.50	16.00
50	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	5	27.50	16.00
100	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	5	27.50	16.00

Auxiliary Tests					Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)					1	0.9		
Equality of variance cannot be confirmed								
The control means are not significantly different (p = 1.00)					0	2.3060041		
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU				
Steel's Many-One Rank Test Treatments vs ERM-Control	**100**	**>100**		1				

Pimephales promelas 96-Hour Acute Toxicity Test for Non-treated Emory River Dredge Plume March 24 – 28, 2010

Acute Fathead Minnow Test-96 Hr Survival

Start Date:	3/24/2010	Test ID:	6072	Sample ID:	KIF, Emory River Dredge Plume
End Date:	3/28/2010	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	Non-treated Grab
Sample Date:	3/23/2010	Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	PP-Pimephales promelas
Comments:	**1 Dredge Plume grab sample for day 0 (initiation) and day 2 (renewal)**				

Conc-%	1	2	3	4	5
MHSW-Control	1.0000	1.0000	1.0000	1.0000	1.0000
ERM-Control	0.5000	1.0000	1.0000	0.9000	1.0000
6.25	0.9000	1.0000	0.7000	0.9000	1.0000
12.5	1.0000	1.0000	1.0000	0.6000	1.0000
25	0.9000	0.8000	1.0000	1.0000	1.0000
50	1.0000	1.0000	1.0000	1.0000	0.9000
100	1.0000	1.0000	0.9000	1.0000	1.0000

			Transform: Arcsin Square Root					Rank	1-Tailed
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical
MHSW-Control	1.0000	1.1364	1.4120	1.4120	1.4120	0.000	5		
ERM-Control	***0.8800**	1.0000	1.2541	0.7854	1.4120	21.637	5		
6.25	0.9000	1.0227	1.2627	0.9912	1.4120	13.643	5	26.00	16.00
12.5	0.9200	1.0455	1.3068	0.8861	1.4120	17.998	5	30.00	16.00
25	0.9400	1.0682	1.3184	1.1071	1.4120	10.436	5	28.00	16.00
50	0.9800	1.1136	1.3794	1.2490	1.4120	5.284	5	30.50	16.00
100	0.9800	1.1136	1.3794	1.2490	1.4120	5.284	5	30.50	16.00

***Invalid test due to mortality in ERM-Control. Mortality was attributed to pathogenic bacteria and fungus.**

Auxiliary Tests					Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)					0.8070448	0.9	-1.6415283	2.4059093
Bartlett's Test indicates equal variances (p = 0.08)					9.8089638	15.086272		
The control means are not significantly different (p = 0.23)					1.3013338	2.3060041		
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU				
Steel's Many-One Rank Test	**100**	**>100**		1				
Treatments vs ERM-Control	**INVALID TEST**							

Pimephales promelas 96-Hour Acute Toxicity Test for UV-treated Emory River Dredge Plume
March 24 – 28, 2010

Acute Fathead Minnow Test-96 Hr Survival

Start Date:	3/24/2010	Test ID:	6072	Sample ID:	KIF, Emory River Dredge Plume
End Date:	3/28/2010	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	UV-treated Grab
Sample Date:	3/23/2010	Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	PP-Pimephales promelas
Comments:	**1 Dredge Plume grab sample for day 0 (initiation) and day 2 (renewal)**				

Conc-%	1	2	3	4	5
MHSW-Control	1.0000	1.0000	1.0000	1.0000	1.0000
ERM-Control	1.0000	1.0000	1.0000	1.0000	1.0000
6.25	1.0000	1.0000	1.0000	1.0000	1.0000
12.5	1.0000	1.0000	1.0000	1.0000	1.0000
25	1.0000	1.0000	1.0000	1.0000	1.0000
50	1.0000	1.0000	1.0000	1.0000	1.0000
100	1.0000	1.0000	1.0000	1.0000	1.0000

			Transform: Arcsin Square Root					Rank	1-Tailed
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical
MHSW-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	5		
ERM-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	5		
6.25	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	5	27.50	16.00
12.5	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	5	27.50	16.00
25	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	5	27.50	16.00
50	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	5	27.50	16.00
100	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	5	27.50	16.00

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	1	0.9		
Equality of variance cannot be confirmed				
The control means are not significantly different (p = 1.00)	0	2.3060041		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	**100**	**>100**		1
Treatments vs ERM-Control				

APPENDIX B

ToxCalc™ v5.0.23F Statistics Report
for *Ceriodaphnia dubia* and *Pimephales promelas*
96-hour Acute Toxicity Tests of TVA Kingston Fossil Plant, Stilling Pond Outfall 001
March 24 – 28, 2010

Environmental Testing Solutions, Inc. Project # 6072

Ceriodaphnia dubia 96-Hour Acute Toxicity Test for Non-treated Stilling Pond Outfall 001
March 24 – 28, 2010

Acute Daphnid Test-96 Hr Survival					
Start Date:	3/24/2010	Test ID:	6072	Sample ID:	KIF, Stilling Pond Outfall 001
End Date:	3/28/2010	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	Non-treated 24-hour Composite
Sample Date:	3/23/2010	Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	CD-Ceriodaphnia dubia
Comments:	**1 Stilling Pond Effluent composite sample for day 0 (initiation) and day 2 (renewal)**				

Conc-%	1	2	3	4	5
MHSW-Control	1.0000	1.0000	1.0000	1.0000	1.0000
ERM-Control	1.0000	1.0000	1.0000	1.0000	1.0000
6.25	1.0000	1.0000	1.0000	1.0000	1.0000
12.5	1.0000	1.0000	1.0000	1.0000	1.0000
25	1.0000	1.0000	1.0000	1.0000	1.0000
50	1.0000	1.0000	1.0000	1.0000	1.0000
100	1.0000	1.0000	1.0000	1.0000	1.0000

			Transform: Arcsin Square Root					Rank	1-Tailed
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical
MHSW-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	5		
ERM-Control	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	5		
6.25	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	5	27.50	16.00
12.5	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	5	27.50	16.00
25	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	5	27.50	16.00
50	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	5	27.50	16.00
100	1.0000	1.0000	1.3453	1.3453	1.3453	0.000	5	27.50	16.00

Auxiliary Tests					Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)					1	0.9		
Equality of variance cannot be confirmed								
The control means are not significantly different (p = 1.00)					0	2.3060041		
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU				
Steel's Many-One Rank Test	**100**	**>100**		1				
Treatments vs ERM-Control								

Pimephales promelas 96-Hour Acute Toxicity Test for Non-treated Stilling Pond Outfall 001
March 24 – 28, 2010

Acute Fathead Minnow Test-96 Hr Survival					
Start Date:	3/24/2010	Test ID:	6072	Sample ID:	KIF, Stilling Pond Outfall 001
End Date:	3/28/2010	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	Non-treated 24-hour Composite
Sample Date:	3/23/2010	Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	PP-Pimephales promelas
Comments:	**1 Still Pond Effluent composite sample for day 0 (initiation) and day 2 (renewal)**				

Conc-%	1	2	3	4	5
MHSW-Control	1.0000	1.0000	1.0000	1.0000	1.0000
ERM-Control	0.6000	1.0000	1.0000	1.0000	1.0000
6.25	0.9000	1.0000	1.0000	0.8000	1.0000
12.5	1.0000	0.8000	1.0000	1.0000	1.0000
25	1.0000	1.0000	0.7000	1.0000	1.0000
50	1.0000	1.0000	1.0000	1.0000	1.0000
100	1.0000	1.0000	1.0000	1.0000	1.0000

			Transform: Arcsin Square Root					Rank	1-Tailed
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical
MHSW-Control	1.0000	1.0870	1.4120	1.4120	1.4120	0.000	5		
ERM-Control	0.9200	1.0000	1.3068	0.8861	1.4120	17.998	5		
6.25	0.9400	1.0217	1.3184	1.1071	1.4120	10.436	5	26.00	16.00
12.5	0.9600	1.0435	1.3510	1.1071	1.4120	10.092	5	28.00	16.00
25	0.9400	1.0217	1.3278	0.9912	1.4120	14.174	5	28.00	16.00
50	1.0000	1.0870	1.4120	1.4120	1.4120	0.000	5	30.00	16.00
100	1.0000	1.0870	1.4120	1.4120	1.4120	0.000	5	30.00	16.00

Auxiliary Tests					Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)					0.7184442	0.9	-1.9864012	3.5363989
Equality of variance cannot be confirmed								
The control means are not significantly different (p = 0.35)					1	2.3060041		
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU				
Steel's Many-One Rank Test	**100**	**>100**		1				
Treatments vs ERM-Control								

Pimephales promelas 96-Hour Acute Toxicity Test for UV-treated Stilling Pond Outfall 001
March 24 – 28, 2010

Acute Fathead Minnow Test-96 Hr Survival

Start Date:	3/24/2010	Test ID:	6072	Sample ID:	KIF, Stilling Pond Outfall 001
End Date:	3/28/2010	Lab ID:	ETS-Envir. Testing Sol.	Sample Type:	UV-treated 24-hour Composite
Sample Date:	3/23/2010	Protocol:	ACUTE-EPA-821-R-02-012	Test Species:	PP-Pimephales promelas
Comments:	**1 Still Pond Effluent composite sample for day 0 (initiation) and day 2 (renewal)**				

Conc-%	1	2	3	4	5
MHSW-Control	1.0000	1.0000	1.0000	1.0000	1.0000
ERM-Control	1.0000	1.0000	1.0000	1.0000	1.0000
6.25	1.0000	1.0000	1.0000	1.0000	1.0000
12.5	1.0000	1.0000	1.0000	1.0000	1.0000
25	1.0000	1.0000	1.0000	1.0000	1.0000
50	1.0000	1.0000	1.0000	1.0000	1.0000
100	1.0000	1.0000	1.0000	1.0000	1.0000

			Transform: Arcsin Square Root					Rank	1-Tailed
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical
MHSW-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	5		
ERM-Control	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	5		
6.25	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	5	27.50	16.00
12.5	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	5	27.50	16.00
25	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	5	27.50	16.00
50	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	5	27.50	16.00
100	1.0000	1.0000	1.4120	1.4120	1.4120	0.000	5	27.50	16.00

Auxiliary Tests					Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)					1	0.9		
Equality of variance cannot be confirmed								
The control means are not significantly different (p = 1.00)					0	2.3060041		
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU				
Steel's Many-One Rank Test	**100**	**>100**		1				
Treatments vs ERM-Control								